Quantitative analysis in chromatography

Calibration

Calibration is the dependence of analytical signal versus concentration of the analyte

Example of analytical signals:

1) consumption of titration reagent;
2) absorbance
3) potential
4) electric current
5) peak area
6) abundance
7) fluorescence intensity

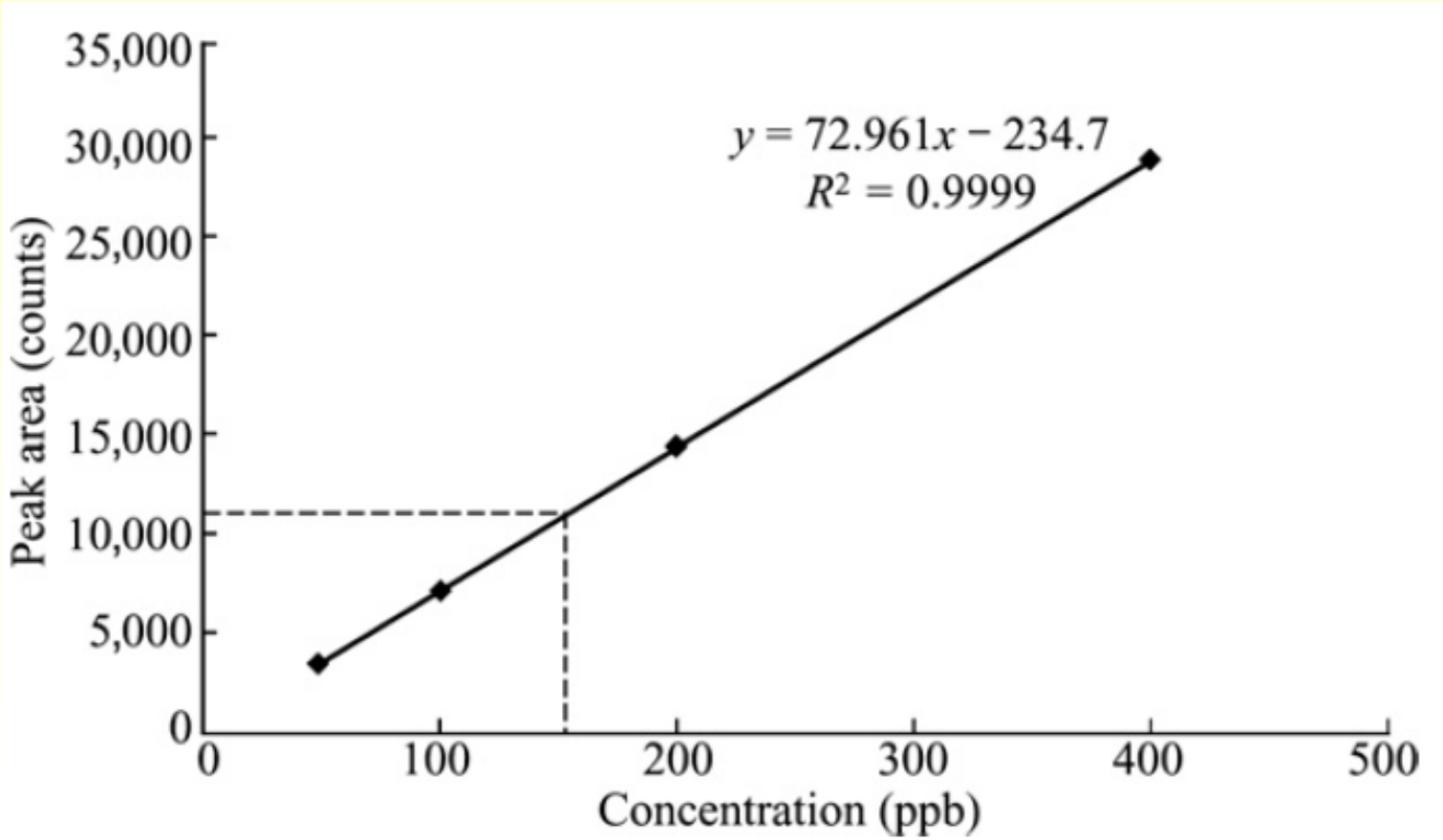
Calibration methods

External standard calibration (classic and most widely applied method)

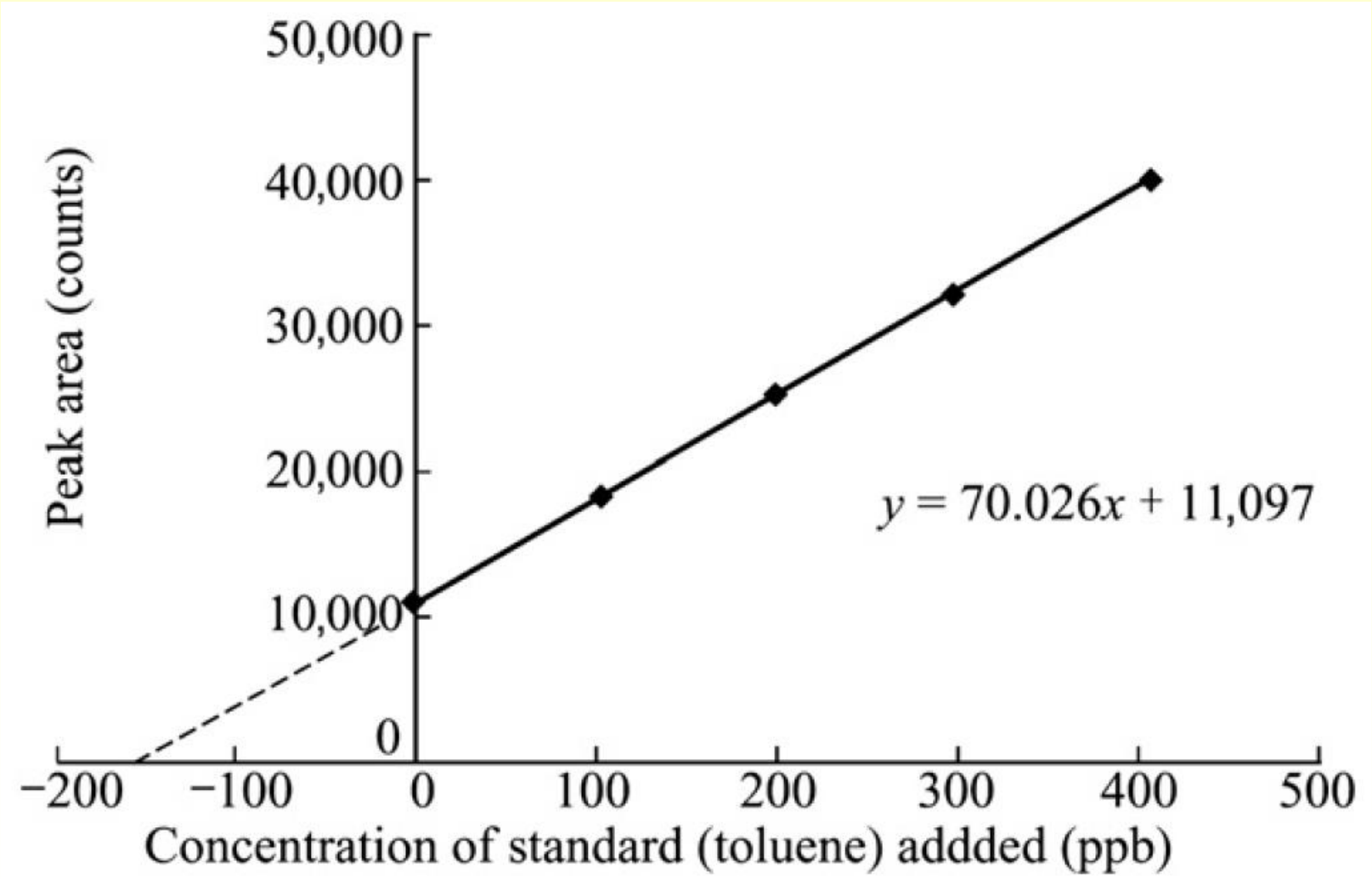
Internal standard calibration (including isotope dilution)

Standard addition

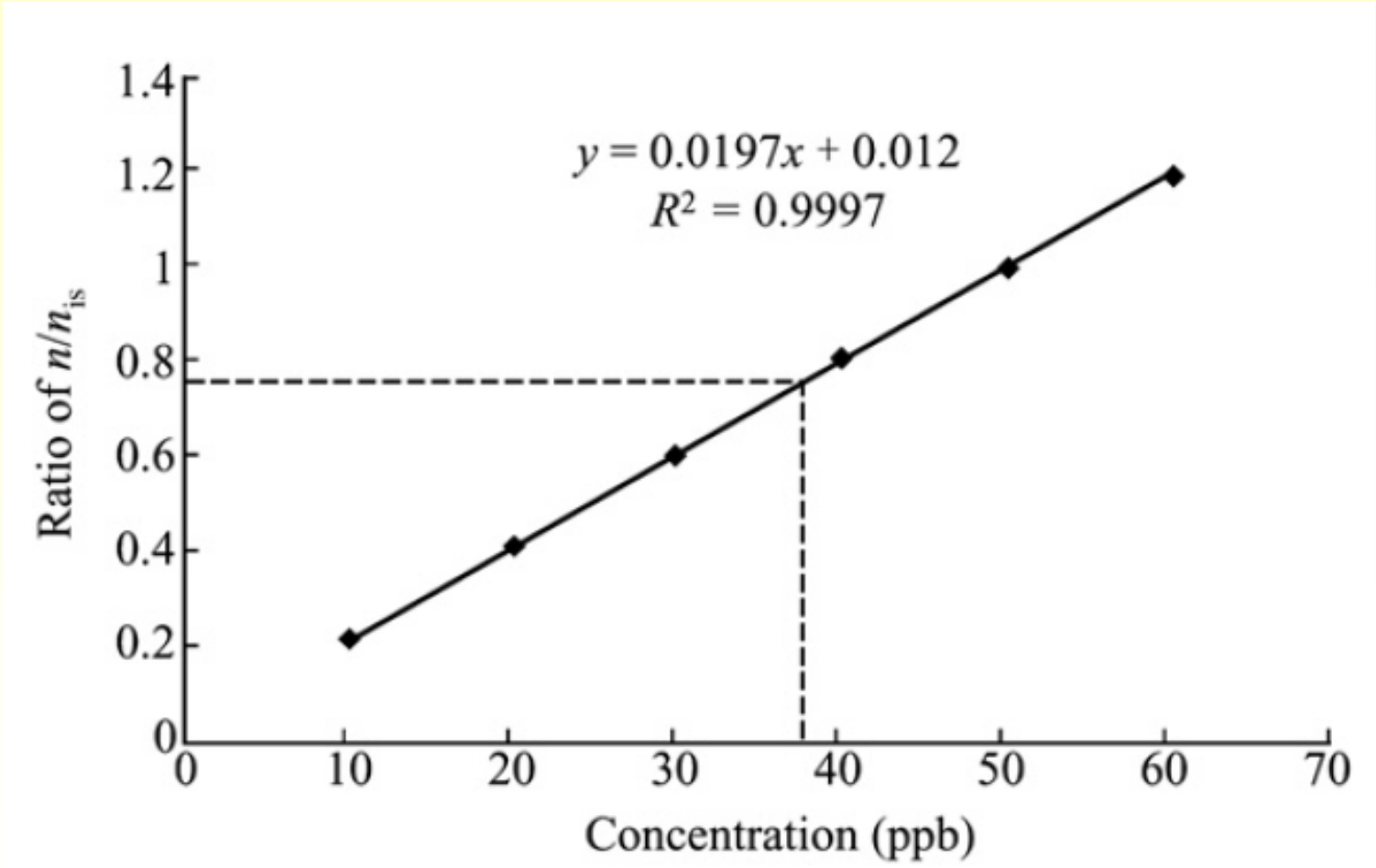
External standard calibration



Calibration using standard addition



Internal standard calibration



Internal standard (IS) analysis

Prepare calibration standards having different analyte concentration

Spike standards with the same solution of IS

Spike all analyzed samples with the same solution of IS

Analyze calibration and studied samples

Calculate signals of analyte and IS

Internal standard (IS) analysis

Calculate S_a/S_{is} for every sample

Build the calibration plot $S_a/S_{is} = f(C_a)$: $\mathbf{S_a/S_{is} = a \cdot C_a}$

Determine analyte concentration using the plot: $\mathbf{C_a = (S_a/S_{is}) / a}$

Exercise

You have to determine concentration of diuron (pesticide) in a water sample by LC-MS. Caffeine was used as the internal standard added to all samples at concentration 100 µg/L. Analysis of calibration samples with concentrations 1, 3, 5, 10, 30 and 50 µg/L gave the following peak areas: 61, 266, 439, 712, 2344, 3999 arbitrary units. Peak area of caffeine were 5569, 8014, 8014, 6425, 7036, 7280 a.u.

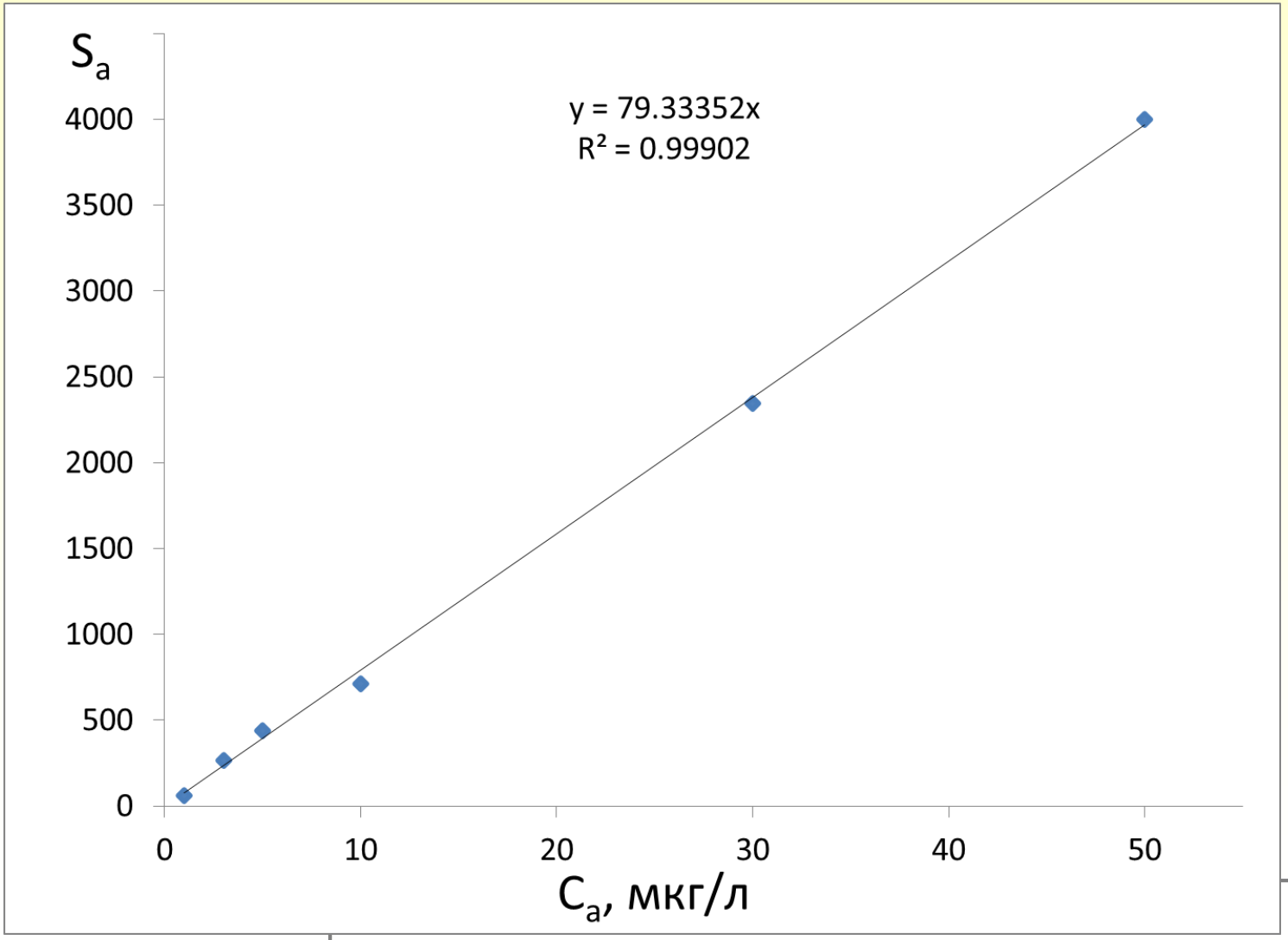
At chromatogram of the analyzed sample, peak areas of diuron and caffeine were 3649 and 7225 a.u., respectively.

Calculate diuron concentrations in analyzed sample by external and internal standard methods.

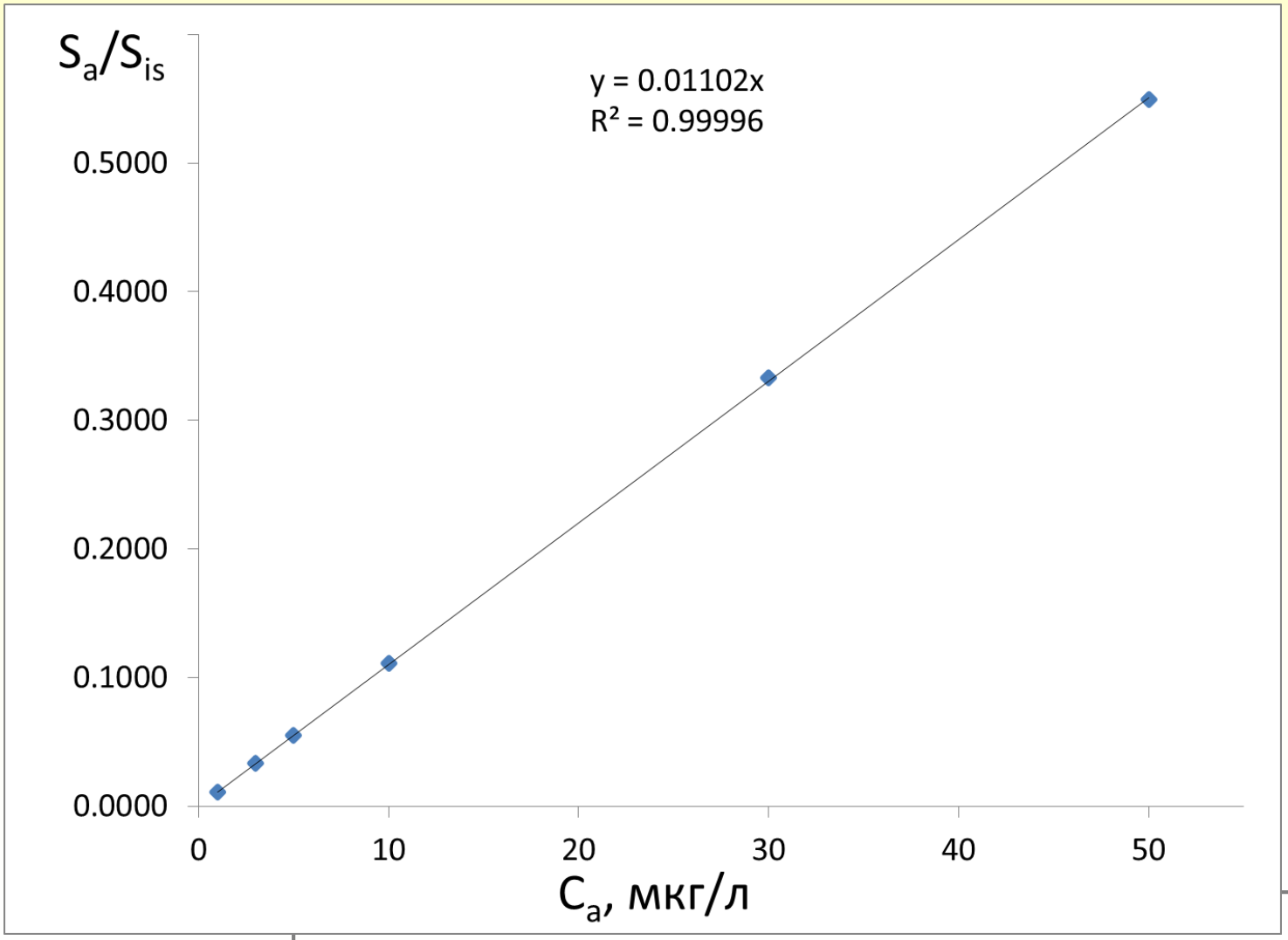
Calculations

C (diuron), µg/L	S (diuron), a.u.	S (caffeine), a.u.	S (diuron)/ S (caffeine)
1	61	5569	0.0110
3	266	8014	0.0332
5	439	8014	0.0548
10	712	6425	0.1108
30	2344	7036	0.3331
50	3999	7280	0.5493

Calibration (ES)



Calibration (IS)



Calculations

H5 | {=ЛИНЕЙН(E5:E10,B5:B10,ЛОЖЬ,ИСТИНА)}

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2			**Данные**					**Расчеты**						
3	**Калибровка**							Внутренний стандарт				Внешний стандарт		
4		C (диурон), мкг/л	S (диурон), у.е.	S (кафеин), у.е.	S(диурон)/ S (кафеин)			Тангенс	Отрезок			Тангенс	Отрезок	
5		1	61	5569	0.0110		Параметр	0.01101911	0			79.33	0	
6		3	266	8014	0.0332		Станд. отклон. (СО)	0.000024	#Н/Д			0.82	#Н/Д	
7		5	439	8014	0.0548		R^2	1.0000	0.00140138	Sy		0.9995	48.721202	Sy
8		10	712	6425	0.1108									
9		30	2344	7036	0.3331		Относит. СО, %	0.21				1.03		
10		50	3999	7280	0.5493		**Уравнение: S_a/S_{is} = (0,011019±0,000024) C**					**S = (79,33±0,82) C**		
11														
12	**Анализ**													
13			3649	7225	0.5051		**Концентрация диурона, мкг/л**	**45.8**				**46.0**		

Advantages of IS over ES

Control of analyte losses during sample preparation

Control of volumes is not required

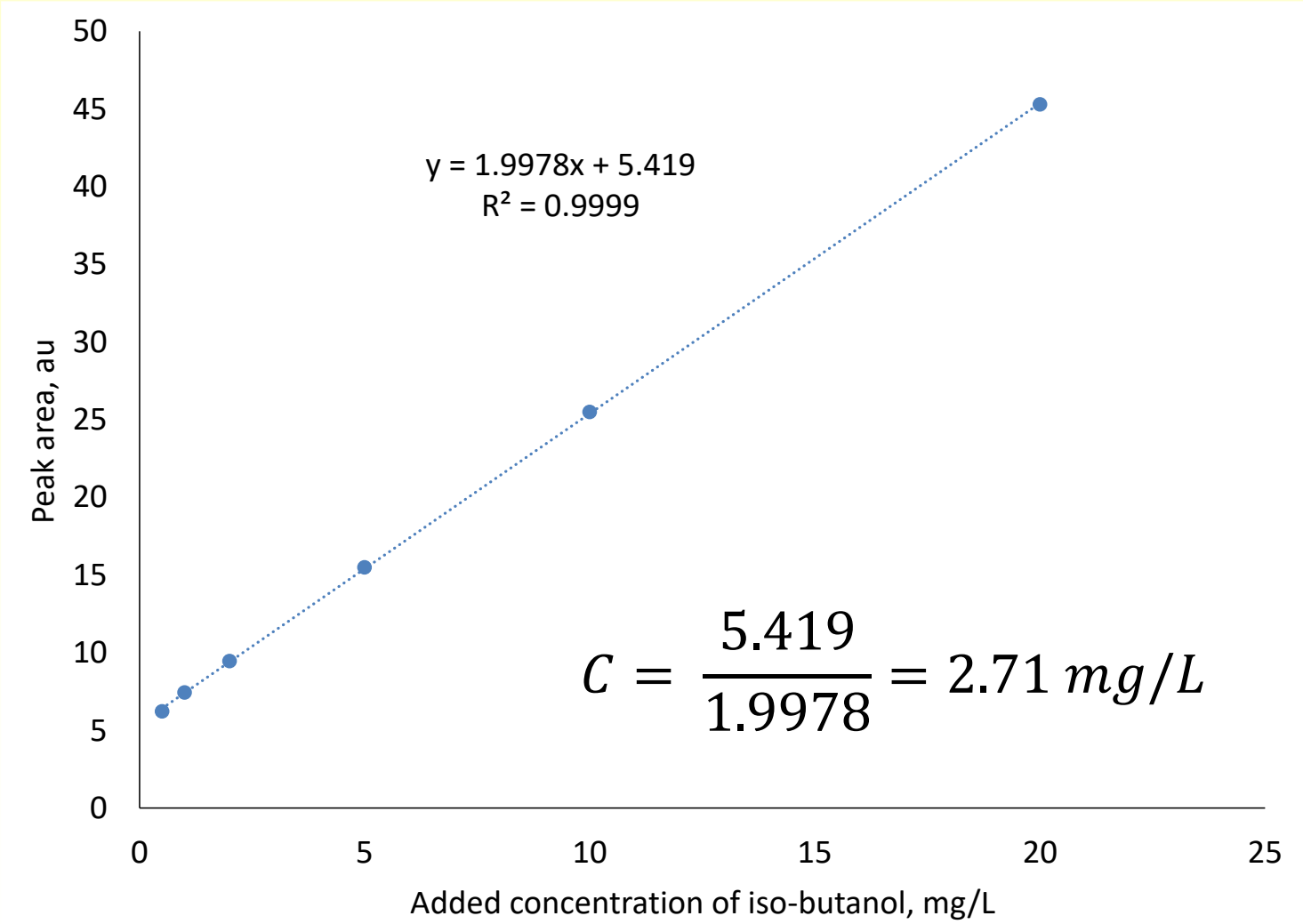
Control of instrument sensitivity

Higher accuracy and precision of the method

Exercise

Brandy sample was analyzed for the concentration of iso-butanol using standard addition method. Standard additions were 0.50; 1.00; 2.00; 5.00; 10.0 and 20.0 mg/L. Peak areas of iso-butanol peaks were 6.23; 7.44; 9.46; 15.5; 25.5 и 45.3 arbitrary units. Calculate the concentration of iso-butanol in the analyzed sample.

Calibration (SA)



$$C = \frac{5.419}{1.9978} = 2.71\ mg/L$$

MS Excel calculations

C ad, mg/L	S, au			Slope	Intercept	
0.50	6.23		Value	1.998	5.419	
1.00	7.44		SD	0.0	0.1	
2.00	9.46		R2	0.9999	0.1	Sy
5.00	15.5		RSD %	0.4	1.3	
10.00	25.5		C mg/L	2.71	1.3	0.04
20.00	45.3					

Advantages of calibration methods

External standard – simple and fast

Internal standard – very precise and fast

Standard addition - matrix effect is controlled